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Biochemical, Physiochemical, and Microbial Changes of Chemically Treated Cabbage Slaw During Storage

L. M. CHEN

A. C. PENG

OHIO AGRICULTURAL RESEARCH AND DEVELOPMENT CENTER
U. S. 250 and OHIO 83 SOUTH
WOOSTER, OHIO

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L. M. CHEN and A. C. PENG

SUMMARY

Cabbage slaws were treated with acid, sorbate, and metabisulfite. Their pH, total acidity, phenol content, enzyme activity, microbial changes, and oxygen depletion were studied. Keeping quality was also evaluated. A correlation between the color change and polyphenol oxidase specific activity was observed. Citric acid, potassium sorbate, and sodium metabisulfite retarded discoloration of cabbage slaw. The concentration of sodium metabisulfite was critical. A 350 ppm in 0.2% citric acid and 300 ppm in 0.2% citric acid/0.1% potassium sorbate extended the shelf life to 3 weeks in terms of color, flavor, texture, and microbial quality. Generally, total acidity, phenol content, and microbial counts increased with storage time, while pH, enzyme activity, color, and oxygen decreased. The treated samples maintained acceptable flavor and texture throughout.

INTRODUCTION

It has been shown that notable chemical and physical changes are associated with deterioration of fresh vegetables. These include changes in pH, color, texture, and aroma. Discoloration is particularly significant on the cut surfaces of cabbage and coleslaw as well as other vegetables and fruits. The chemical system responsible for this darkening may be related to an enzyme, namely polyphenol oxidase, and its reaction with a phenolic substrate in the presence of molecular oxygen. This reaction is referred to as enzymatic browning (17).

Enzymatic browning of vegetables and fruits has been of great interest in the food industry. The characteristics of polyphenol oxidase (PPO) and control of browning have been reviewed (17, 26). Browning can be reduced by blanching, chilling, addition of sugar, salt, and chemical inhibitors of PPO, or by selecting cultivars of raw material less susceptible to browning and/or raw material at the stage of maturity at which discoloration is at a minimum (14).

Several methods have been suggested to control discoloration in coleslaw. These include: vacuum cooling (10), removal of oxygen from the atmosphere surrounding the cabbage shreds (8, 19), or treatment with acids, reducing reagents, antibiotics, and other chemical inhibitors. Most observations upon treatments were made objectively on

the changes of color (8) and microbial populations (32), or subjective evaluations of appearance by trained panelists (19). Little is known concerning the effect of treatments and storage on changes of pH, total acidity, phenol content, PPO activity, and other physical or chemical properties accompanying discoloration. Knowledge of these changes should be helpful in developing methods for preventing discoloration.

The objective of this research was to study biochemical, physiochemical, and microbial changes occurring in packaged cabbage slaw treated with citric acid, potassium sorbate and potassium metabisulfite during 3 weeks of refrigerated storage. Investigations included pH, total acidity, phenol content, PPO specific activity, microbial studies, oxygen uptake by cabbage slaw, as well as sensory evaluation.

MATERIAL AND METHODS

Preparation of Packaged Cabbage Slaw

Cabbage heads obtained from the wholesale market were inspected, decorded, and shredded. Shredded cabbage was then dipped in the test solutions as a proportion of 1144 g of shreds to 4 L of solution for 2 min. After draining and drying with layers of cheesecloth, cabbage shreds were packed in rigid High-Density polyethylene containers (1.8 L) and stored in the refrigerator at 4° C for 3 weeks. Slaw dressing, which may affect chemical analysis, was not added.

Test solutions in this experiment were: 0.2% citric acid; 0.2% citric acid with 200 ppm, 350 ppm, 500 ppm, 650 ppm, and 800 ppm of sodium metabisulfite, respectively; 0.1% potassium sorbate; 0.1% potassium sorbate with 200 ppm, 350 ppm, 500 ppm, 650 ppm, and 800 ppm of sodium metabisulfite, respectively; 0.2% citric acid with 0.1% potassium sorbate and their combinations with 200 ppm, 300 ppm, 400 ppm, and 500 ppm of sodium metabisulfite. Tater white (L. K. Baker, Columbus, Ohio) and STA white (Archibald & Kendall, Inc., New York, N.Y.) were prepared as specified by the manufacturers. Water dipped samples were used as a control. Four replicates from each differently treated samples were randomly taken at the 1st, 7th, 14th, and 21st day of storage for the following objective measurements.

pH and Total Acidity

The glass electrode method (1) was applied to measure the pH and titratable acidity in a sample solution prepared by blending 5 g cabbage shreds with 95 ml of distilled water. Total acidity was expressed as percent titratable citric acid in 50 ml of sample aliquot.

Color Determination

Color determination on acetone-extracted residue of cabbage slaw was performed by Hunter Color Difference Meter D25D3 against a yel-

low standard (L = 77.3, a = —2.0, b = 22.6). Samples were prepared by the method of Francis (8).

Preparation of Acetone Powder and Enzyme Extract

Acetone powder of cabbage slaw and crude enzyme were prepared as described in the previous report (4), except that enzyme extract was not purified.

Assay Procedures

The procedures were the same as that described for PPO activity assay in Hi-Dri cabbage (4).

Protein content of each enzyme extract was determined by the method of Lowry (23).

One unit of enzyme activity is defined as the change in absorbance of 0.001 per min at 410 nm. Specific activity is the units of activity per mg of protein.

Determination of Phenol Content

Phenols in cabbage slaw were extracted by blending 20 g shreds with 80 ml absolute methanol for 2 min, and boiling for 5 min. The sample slurry was then filtered and washed with hot methanol twice to make a total volume of 100 ml.

Total phenolic concentration was determined by Folin-Denis method (1) against standard tannic solutions.

Determination of Oxygen in the Headspace of Container

This experiment was intended to study the changes of O_2 tension in the headspace of the container which was equipped with a rubber septum on the lid. The headspace of the container was equilibrated with air at the beginning of the storage. Three ml of gas were withdrawn from the headspace at each week and analyzed by a Becker gas chromatograph (Packard Model 417). Nitrogen was used as the carrier gas with a flow rate between 50 to 56 ml per min. The temperatures of column, detector, and injection during operation were 100° C, 160° C, and 170° C, respectively. The percent of O_2 was calculated by comparing the height of O_2 peak of a gas sample to that of a standard gas which contained 20.2% O_2.

Sensory Evaluation

Sensory evaluations of cabbage slaw were performed by six to ten untrained panel members. The samples were graded on the basis of color, flavor, texture and total acceptability, and scored on a scale of 1 to 10; 10 was considered as perfect, 5 was acceptable, and 1 was off.

Determination of Microbial Count

Baco tomato juice agar was selected as media for enumeration of microorganisms in cabbage slaw.

A 10 g sample was weighed into a sterile blender cup with addition of 90 ml citrate-phosphate buffer (pH 6.8). The sample was blended for 2 min. Consecutive serial dilutions from 10^{-2} to 10^{-7} were prepared.

Duplicate plates with 10^{-2} through 10^{-7} dilutions were poured with 12-14 ml of tomato juice agar, allowed to solidify, and incubated at room temperature for 4 days. Preparation and counting were performed in accordance with Standard Methods for the Examination of Dairy Products (2).

Statistical Analysis

All results were statistically analyzed. The significant differences among treated and control sample means were performed by Duncan's multiple range test (28) on the basis of each sampling. The least sig-

TABLE 1.—Calculated Means of pH and Total Acidity of Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

	Storage (Day)							
	1		7		14		21	
Sample†	pH	Percent Total Acidity	pH	Percent Total Acidity	pH	Percent Total Acidity	pH	Percent Total Acidity
1	6.08	0.0047	6.51	0.0043	6.30	0.0050	6.50	0.0047
2	6.10	0.0040	6.31	0.0048	6.50	0.0050	6.50	0.0050
3	6.23	0.0040	6.20	0.0042	6.30	0.0050	6.30	0.0050
4	6.30	0.0060	6.30	0.0067	5.90	0.0067	5.70*	0.0077
5	4.60*	0.0130*	4.50*	0.0130*	4.70*	0.0147*	4.60*	0.0140*
6	4.90*	0.0093*	4.80*	0.0104*	4.90*	0.0100*	4.90*	0.0113*
7	4.90*	0.0120*	4.85*	0.0130*	4.71*	0.0140*	4.70*	0.0170*
8	6.30	0.0050	6.50	0.0040	6.51	0.0047	4.80*	0.0147*
9	6.30	0.0050	6.60	0.0041	6.50	0.0041	6.20	0.0047
10	6.50	0.0050	6.50	0.0040	6.50	0.0050	6.50	0.0047
11	6.40	0.0045	6.40	0.0040	6.55	0.0050	6.50	0.0050
12	6.40	0.0050	6.42	0.0050	6.40	0.0050	6.40	0.0053
13	6.50	0.0047	6.50	0.0050	6.60	0.0051	6.50	0.0051

†1 – water-dipped control
2 – 0.2 % citric acid
3 – 0.2 % citric acid/200 ppm sodium metabisulfite ($Na_2S_2O_5$)
4 – 0.2 % citric acid/350 ppm $Na_2S_2O_5$
5 – 0.2 % citric acid/500 ppm $Na_2S_2O_5$
6 – 0.2 % citric acid/650 ppm $Na_2S_2O_5$
7 – 0.2 % citric acid/800 ppm $Na_2S_2O_5$
8 – 0.1 % potassium sorbate
9 – 0.1 % potassium sorbate/200 ppm $Na_2S_2O_5$
10 – 0.1 % potassium sorbate/350 ppm $Na_2S_2O_5$
11 – 0.1 % potassium sorbate/500 ppm $Na_2S_2O_5$
12 – 0.1 % potassium sorbate/650 ppm $N_2S_2O_5$
13 – 0.1 % potassium sorbate/800 ppm $Na_2S_2O_5$
*Significantly different from control samples as calculated by Duncan's multiple range test.

nificant difference (LSD) was calculated to evaluate differences among treatments (21).

RESULTS AND DISCUSSION

Changes in pH and Total Acidity upon Treatment and Storage

The pH values in those samples treated with 500, 650, and 800 ppm $Na_2S_2O_5$ in citric acid solutions and with 400 and 500 ppm $Na_2S_2O_5$ in the solutions containing both citric acid and potassium sorbate as well as STA were between 4.6 and 5.4 (Tables 1 and 2), which were much lower than the control and other treated samples. The original pH values of these dip solutions were between 2.6 and 2.7 at which only bisulfite (HSO_3^-) and undissociated sulfurous acid existed (15). The higher the concentration of $Na_2S_2O_5$ in the solution, the greater the amount of acid sulfite which could penetrate into tissues, with a consequent reduction of pH in the cabbage shreds. Accompanying the pH changes, tissues softened and plasmolysis occurred, releasing cellular fluids. These observations, as seen in heavily sulfured dried fruits, indicated the degradation of cell walls and intercellular layers as well as an alternation to the osomotic equilibrium within the tissues (27).

TABLE 2.—Calculated Means of pH and Total Acidity of Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

	Storage (Day)							
	1		7		14		21	
Sample†	pH	Percent Total Acidity	pH	Percent Total Acidity	pH	Percent Total Acidity	pH	Percent Total Acidity
1	6.63	0.0039	6.40	0.0039	6.30	0.0058	6.30	0.0058
2	6.05*	0.0058	6.03	0.0058	5.93	0.0064	5.35*	0.0109*
3	6.35	0.0052	6.35	0.0052	6.23	0.0071	6.25	0.0071
4	6.50	0.0052	6.30	0.0064	6.10	0.0071	6.15	0.0071
5	5.38*	0.0093*	5.35*	0.0090*	5.28*	0.0096*	4.18*	0.0132*
6	5.30*	0.0096*	5.35*	0.0090*	5.28*	0.0096*	4.20*	0.0135*
7	6.35	0.0055	6.23	0.0071	6.05	0.0077	6.05	0.0090
8	5.40*	0.0141*	5.30*	0.0148*	5.33*	0.0141*	5.30*	0.0141*

†1 — water-dipped control
2 — 0.2 % citric acid/0.1 % potassium sorbate
3 — 0.2 % citric acid/0.1 % potassium sorbate/200 ppm $Na_2S_2O_5$
4 — 0.2 % citric acid/0.1 % potassium sorbate/300 ppm $Na_2S_2O_5$
5 — 0.2 % citric acid/0.1 % potassium sorbate/400 ppm $Na_2S_2O_5$
6 — 0.2 % citric acid/0.1 % potassium sorbate/500 ppm $Na_2S_2O_5$
7 — Tater white (commercial whitener)
8 — STA white (commercial bleaching reagent, ingredients unknown). Samples treated with STA white contained more than 1,000 ppm SO_2.
*Significantly different from control samples as calculated by Duncan's multiple range test.

Changes in total acidity upon treatment mostly related to the changes in pH values; where there was a decrease in pH, there was an increase in total acidity.

At pH level of 4.8 to 7.3 in the dip solutions containing potassium sorbate and different concentrations of $Na_2S_2O_5$, sulfite existed as sulfite (SO_3^{2-}) and bisulfite (HSO_3^-) ions but not undissociated sulfurous acid (H_2SO_3) which is the most active form of sulfur dioxide. As a result, potassium sorbate with $Na_2S_2O_5$ did not influence pH and total acidity in packaged cabbage slaw (Table 1).

The effect of storage was only seen in samples treated with potassium sorbate (sample 8 in Table 1) and samples treated with citric acid, potassium sorbate, and their combinations with 400, 500 ppm $Na_2S_2O_5$ (samples 2, 5, 6 in Table 2). The onset of fermentation is suspected to

TABLE 3.—Calculated Means of Phenolic Content (mg Tannic Acid/ml Sample) of Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

	Storage (Day)				
Sample†	1	7	14	21	△‡
1	0.030	0.040	0.040	0.072	0.042
2	0.030	0.036	0.041	0.060	0.030
3	0.030	0.037	0.040	0.059	0.029
4	0.032	0.040	0.040	0.041*	0.009
5	0.060*	0.070*	0.071*	0.086*	0.026
6	0.058*	0.058*	0.070*	0.072	0.014
7	0.070*	0.070*	0.058*	0.086*	0.016
8	0.025	0.040	0.041	0.060	0.035
9	0.020	0.040	0.041	0.060	0.040
10	0.031	0.030	0.040	0.040*	0.009
11	0.030	0.030	0.040	0.040*	0.010
12	0.040*	0.043	0.060*	0.060	0.020
13	0.050*	0.050*	0.060*	0.060	0.010

†1 – water-dipped control
2 – 0.2 % citric acid
3 – 0.2 % citric acid/200 ppm $Na_2S_2O_5$
4 – 0.2 % citric acid/350 ppm $Na_2S_2O_5$
5 – 0.2 % citric acid/500 ppm $Na_2S_2O_5$
6 – 0.2 % citric acid/650 ppm $Na_2S_2O_5$
7 – 0.2 % citric acid/800 ppm $Na_2S_2O_5$
8 – 0.1 % potassium sorbate
9 – 0.1 % potassium sorbate/200 ppm $Na_2S_2O_5$
10 – 0.1 % potassium sorbate/350 ppm $Na_2S_2O_5$
11 – 0.1 % potassium sorbate/500 ppm $Na_2S_2O_5$
12 – 0.1 % potassium sorbate/650 ppm $Na_2S_2O_5$
13 – 0.1 % potassium sorbate/800 ppm $Na_2S_2O_5$
‡Amount of change in phenolic content after 21 days of storage.
*Significantly different from control samples as calculated by Duncan's multiple range test.

account for the sharp decrease in pH values and increase in total acidity. The change influenced in other than these treatments was likely to be smaller than the sampling or analytical errors.

Effects of Treatment and Storage on Phenolic Content

Initial phenolic content of packaged cabbage slaw was higher in the samples with higher residual sulfur dioxide such as samples 5, 6, 7, 12, and 13 in Table 3 and samples 6 and 8 in Table 4. Sulfur dioxide is known to reduce the Folin-Denis reagent (33). Joslyn and Goldstein (16) have shown, under their test conditions, that a level of sulfur dioxide in the test mixture from 3.2 μg to 6.2 μg would result in appreciable interference in determining the phenolic content. In this experiment, citric acid/800 ppm $Na_2S_2O_5$ and STA white treated samples contained 3.4 μg and 5.8 μg in the test mixture, respectively, at which this interference could be apparent.

Phenolic content of packaged cabbage slaw increased as storage duration increased regardless of treatment; the increase was particularly significant in the control (Tables 3 and 4). Mondy *et al.* (29) attributed this increase to the synthesis of chlorogenic acid in the disks of potato tubers when they were stored at 4.5° C. The availability of oxygen, glucose, quinic acid, and moisture level would stimulate the

TABLE 4.—Calculated Means of Phenolic Content (mg Tannic Acid/ml Sample) of Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

Sample†	Storage (Day)				
	1	7	14	21	△‡
1	0.036	0.040	0.054	0.065	0.029
2	0.035	0.045	0.042	0.059	0.024
3	0.033	0.041	0.043	0.040	0.007
4	0.042	0.044	0.046	0.055	0.013
5	0.040	0.041	0.042	0.048	0.008
6	0.059*	0.061*	0.068*	0.078*	0.019
7	0.042	0.054*	0.055	0.055	0.013
8	0.095*	0.100*	0.125*	0.143*	0.048

†1 — water-dipped control
2 — 0.2 % citric acid/0.1 % potassium sorbate
3 — 0.2 % citric acid/0.1 % potassium sorbate/200 ppm $Na_2S_2O_5$
4 — 0.2 % citric acid/0.1 % potassium sorbate/300 ppm $Na_2S_2O_5$
5 — 0.2 % citric acid/0.1 % potassium sorbate/400 ppm $Na_2S_2O_5$
6 — 0.2 % citric acid/0.1 % potassium sorbate/500 ppm $Na_2S_2O_5$
7 — Tater white
8 — STA white
‡Amount of change in phenolic content after 21 days of storage.
*Significantly different from control samples as calculated by Duncan's multiple range test.

synthesis of phenolic compounds (6, 39). Cell damage of cabbage tissues might bring about the accumulation of phenols as was observed in virus-infected peaches (11), pear (31), and in mechanically injured potatoes (13), as well as result in increasing the extractability of phenols by solvent.

Changes in PPO Specific Activity of Treated Cabbage Slaw During Storage

It has been shown that PPO specific activity from fresh homogenate of avocado was correlated well with that in acetone powder prepared from fresh tissues (18). The homogenates of packaged cabbage slaw in this study exhibited a lower PPO specific activity (prior experiment). Some factors which could be involved are: 1) homogenates might only provide partial enzyme source, 2) an error caused by unequal particle

TABLE 5.—Calculated Means of Specific Activity (Units/mg Protein) of Polyphenol Oxidase in Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

	Storage (Day)				
Sample†	1	7	14	21	△‡
1	45.1	37.3	30.1	25.3	—19.8
2	43.9	40.2	35.7*	29.8*	—14.1
3	42.8	39.8	34.4*	28.5*	—14.3
4	46.7	44.2*	40.3*	35.6*	—11.1
5	46.6	43.8*	40.2*	37.0*	—11.6
6	47.8*	45.1*	41.2*	38.8*	— 9.0
7	48.7*	45.3*	40.1*	37.9*	—10.8
8	44.3	39.1	34.6*	28.6*	—15.7
9	43.1	39.3	33.4*	27.9*	—15.2
10	45.2	40.2	34.8*	29.6*	—15.6
11	46.8	41.2	35.3*	30.1*	—16.7
12	47.1	44.5*	35.4*	31.1*	—16.0
13	47.4*	42.6*	36.2*	31.2*	—16.2

†1 – water-dipped control
2 – 0.2 % citric acid
3 – 0.2 % citric acid/200 ppm $Na_2S_2O_5$
4 – 0.2 % citric acid/350 ppm $Na_2S_2O_5$
5 – 0.2 % citric acid/500 ppm $Na_2S_2O_5$
6 – 0.2 % citric acid/650 ppm $Na_2S_2O_5$
7 – 0.2 % citric acid/800 ppm $Na_2S_2O_5$
8 – 0.1 % potassium sorbate
9 – 0.1 % potassium sorbate/200 ppm $Na_2S_2O_5$
10 – 0.1 % potassium sorbate/350 ppm $Na_2S_2O_5$
11 – 0.1 % potassium sorbate/500 ppm $Na_2S_2O_5$
12 – 0.1 % potassium sorbate/650 ppm $Na_2S_2O_5$
13 – 0.1 % potassium sorbate/800 ppm $Na_2S_2O_5$
‡Amount of change in specific activity after 21 days of storage.
*Significantly different from the control samples as calculated by Duncan's multiple range test.

distribution in the reaction mixture which often occurred during the measurements, and 3) the presence of inhibitory substances for PPO interfered with the measurements. A recent report also indicated a 20-fold increase in PPO activity in extracts of acetone powder over that from fresh extracts of peaches (7). Therefore, extracts of acetone powder as the enzyme source were suggested in this study.

Results showed that the concentration of metabisulfite affected the initial PPO specific activity in packaged cabbage slaw (Tables 5 and 6). Metabisulfite has been used to facilitate the extraction of active enzyme from plant tissues (22). As a result, higher initial specific activity of PPO was observed in the samples with higher residual sulfur dioxide, such as samples 6, 7, and 13 in Table 5 and samples 7 and 8 in Table 6.

Specific activity of PPO, in general, decreased with time, and the extent of decrease was greater in the control than in the treated samples (Tables 5 and 6). Assuming the enzyme concentration was constant during refrigerated storage, the decrease could be indicative of the occurrence of enzymatic browning in cabbage slaw. Sulfur dioxide is effective in inhibiting enzymatic reactions; consequently, changes in enzyme activity would be smaller in the sulfur-treated samples.

The trend of decreasing PPO activity upon storage was also found in three cultivars of potatoes at 5° C for 60 days (34) and in freeze-dried

TABLE 6.—Calculated Means of Specific Activity (Units/mg Protein) of Polyphenol Oxidase in Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

Sample†	Storage (Day)				
	1	7	14	21	△‡
1	34.0	29.2	22.4	19.5	—14.5
2	33.0	27.9	26.5*	19.6	—13.4
3	36.5	30.0	30.6*	23.6*	—12.9
4	36.2	29.4	29.2*	26.0*	—10.2
5	35.8	33.4*	30.5*	28.5*	— 7.3
6	37.9	35.1*	30.2*	28.8*	— 9.1
7	38.0*	32.9	29.4*	26.7*	—12.3
8	40.7*	40.6*	39.1*	31.4*	— 9.3

†1 — water-dipped control
2 — 0.2 % citric acid/0.1 % potassium sorbate
3 — 0.2 % citric acid/0.1 potassium sorbate/200 ppm $Na_2S_2O_5$
4 — 0.2 % citric acid/0.1 % potassium sorbate/300 ppm $Na_2S_2O_5$
5 — 0.2 % citric acid/0.1 % potassium sorbate/400 ppm $Na_2S_2O_5$
6 — 0.2 % citric acid/0.1 % potassium sorbate/500 ppm $Na_2S_2O_5$
7 — Tater white
8 — STA white
‡Amount of change in specific activity after 21 days of storage.
*Significantly different from control samples as calculated by Duncan's multiple range test.

peaches and bananas at 28° C for 60 days (6). However, PPO activity increased when dates were stored at 49° C for a month (25). The increased activity was suggested as a result of tissue disintegration which released the soluble PPO originating in the particulate fraction, or releasing the particulate fraction to determine whether this discrepancy was due to experimental errors, methodology, individual commodity or chemical treatment of commodity. Further research is certainly required.

Color Changes in Packaged Cabbage Slaw After Treatment and Storage

Based on the prior experiment, the color of cabbage slaw residues after acetone extraction as determined by Hunter L represented the color changes which occurred in the sample. The larger the L value (lightness), the lighter the color of the sample.

TABLE 7.—Calculated Means of Color Scores (Hunter L) of Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

	Storage (Day)				
Sample†	1	7	14	21	△‡
1	75.7	70.2	58.0	55.4	—20.3
2	74.7	75.1*	69.1*	61.9*	—13.3
3	77.4	70.1	66.7*	64.3*	—13.1
4	77.3	69.1	67.9*	65.8*	—12.3
5	79.6*	77.7*	74.1*	70.1*	— 9.5
6	79.4*	76.4*	72.1*	71.3*	— 9.1
7	78.8	77.5*	74.8*	74.2*	— 4.6
8	71.9*	69.6	71.2*	60.2*	—11.7
9	75.8	75.7*	64.1*	63.2*	—12.5
10	75.0	71.3	66.8*	62.8*	—12.2
11	75.8	70.2	67.5*	65.8*	—10.0
12	75.7	72.5	72.1*	66.6*	— 9.0
13	77.2	67.8	68.1*	67.7*	— 9.5

†1 – water-dipped control
2 – 0.2 % citric acid
3 – 0.2 % citric acid/200 ppm sodium metabisulfite ($Na_2S_2O_5$)
4 – 0.2 % citric acid/350 ppm $Na_2S_2O_5$
5 – 0.2 % citric acid/500 ppm $Na_2S_2O_5$
6 – 0.2 % citric acid/650 ppm $Na_2S_2O_5$
7 – 0.2 % citric acid/800 ppm $Na_2S_2O_5$
8 – 0.1 % potassium sorbate
9 – 0.1 % potassium sorbate/200 ppm $Na_2S_2O_5$
10 – 0.1 % potassium sorbate/350 ppm $Na_2S_2O_5$
11 – 0.1 % potassium sorbate/500 ppm $Na_2S_2O_5$
12 – 0.1 % potassium sorbate/650 ppm $Na_2S_2O_5$
13 – 0.1 % potassium sorbate/800 ppm $Na_2S_2O_5$
‡Amount of change in color scores after 21 days of storage.
*Significantly different from control samples as calculated by Duncan's multiple range test.

Chemical treatment did not affect the color values of packaged cabbage slaw at the beginning of the storage except for those samples treated with high concentration of $Na_2S_2O_5$. The significant difference among the treated samples and the control appeared after storage (Tables 7 and 8). This was anticipated since the reagents present in the dip solutions were intended to retard the enzymatic reactions which contributed to the discoloration of cabbage slaw.

Citric acid served as an acidulant in retarding enzymatic browning. A treatment of 0.2% citric acid alone neither reduced the pH value of packaged cabbage slaw (Table 1), nor inhibited discoloration (Table 7) or PPO specific activity (Table 5) when comparing their changes after 3 weeks of storage to the changes which occurred in the control samples. The significance of citric acid used in this experiment is assessed by its synergistic effect with metabisulfite and potassium sorbate.

Potassium sorbate was mainly used as an antimicrobial agent. The mechanism of its function in retarding discoloration was suggested to be inhibition of microbial multiplication which could be the sources of enzymes (5). The inhibition of PPO activity concomitantly with retardation of discoloration by potassium sorbate and its combination with $Na_2S_2O_5$ was not as effective as citric acid and metabisulfite (Tables 5 and 7). A higher concentration of $Na_2S_2O_5$ was required to achieve better results.

TABLE 8.—Calculated Means of Color Scores (Hunter L) of Packaged Cabbage Slaw After 1, 7, 14, and 21 Days of Storage at 4° C.

Sample†	Storage (Day)				
	1	7	14	21	△‡
1	71.7	67.6	61.5	57.9	—13.8
2	72.9	72.7*	69.8*	58.7	—14.2
3	73.6*	71.9*	68.2*	63.0*	—10.6
4	72.7	71.7*	69.7*	66.0	— 6.7
5	72.9	72.1*	65.3*	62.1*	—10.8
6	72.2	71.2*	65.8*	61.6*	—10.6
7	72.8	71.6*	68.9*	66.0*	— 6.8
8	75.2*	72.2*	71.8*	70.0*	— 5.2

†1 — water-dipped control
2 — 0.2% citric acid/0.1% potassium sorbate
3 — 0.2% citric acid/0.1% potassium sorbate/200 ppm $Na_2S_2O_5$
4 — 0.2% citric acid/0.1% potassium sorbate/300 ppm $Na_2S_2O_5$
5 — 0.2% citric acid/0.1% potassium sorbate/400 ppm $Na_2S_2O_5$
6 — 0.2% citric acid/0.1% potassium sorbate/500 ppm $Na_2S_2O_5$
7 — Tater white
8 — STA white
‡Amount of change in color scores after 21 days of storage.
*Significantly different from control samples as calculated by Duncan's multiple range test.

Although the addition of 500-800 ppm $Na_2S_2O_5$ in 0.2% citric acid solutions and STA white solution resulted in the lightest color scores of packaged cabbage slaw, they also brought about high acidity and soft texture as mentioned before. A comparable effectiveness on retarding discoloration was found in the dip solution containing citric acid, potassium sorbate, and 350 ppm $Na_2S_2O_5$. Additive effects of these three reagents were reflected by the changes of color scores and enzyme activities (Tables 6 and 8); yet these treated samples maintained acceptable flavor and texture (Table 9).

A synergistic effect of metabisulfite, salt, citric acid, and ascorbic acid was shown in the Tater white treated samples (Tables 6 and 8). Tater white treatment was as satisfactory as citric acid, potassium sorbate, and 350 ppm $Na_2S_2O_5$ with respect to the changes in color and enzyme activity after storage as well as qualities evaluated by the panels.

In various foods the rate of browning can be due directly to: a) polyphenol oxidase, b) endogenous polyphenol content, or c) a specific combination of both factors (18). The first two have been well documented and several different findings were reported (12, 14, 29, 30, 37, and 38). Data from this study were in favor of the significance of specific activity of PPO on the discoloration in packaged cabbage slaw. A correlation factor of 0.86 was found from the graph plotted of color values from Table 7 vs. specific activity from Table 5, and 0.76 for the graph plotted of color values from Table 8 vs. specific activity from

TABLE 9.—Flavor Scores of Cabbage Slaw by Various Chemical Treatments.*

Treatment	Mean Score
control	8.5
citric acid	7.8
citric acid/500 ppm $Na_2S_2O_5$	5.6**
potassium sorbate	7.7
potassium sorbate/500 ppm $Na_2S_2O_5$	7.8
grand mean	7.48
control	6.1
citric acid/potassium sorbate	8.3
citric acid/potassium sorbate/300 ppm $Na_2S_2O_5$	7.2
Tater white	7.9
STA white	4.2**
grand mean	6.8

*A 10-point hedonic scale was used with 10 as perfect and 1 as off.
**Significant at 1%.

Table 6. Correlation coefficients between phenolic content and color change were insignificant.

Effect of Treatment and Storage on Microbial Count in Cabbage Slaw

In addition to discoloration, the microbial quality of coleslaw, a criterion of probable shelf life, has been of increasing concern to the consuming public. Microbial populations isolated from fresh cabbage slurry were reported as follows: *Brevibacterium, Chromobacterium, Citrobacter, Pseudomonas, Xanthomonas,* and lactic acid bacteria (20). Yeasts and molds only accounted for 1% of the total population. A concentration of citric acid at 0.2% was effective in reducing initial counts in packaged cabbage slaw (Table 10), but the inhibition was not as prolonged as when metabisulfite was present in the dip solution. Both the control and citric acid treated samples discolored at 14 days and spoiled after 21 days of storage.

It is known that acetic acid bacteria, lactic acid bacteria, and many species of molds are more sensitive to sulfur dioxide than yeasts. The

TABLE 10.—Changes of Microbial Count (X 10^5/g) in Treated Cabbage Slaw During the Storage at 4° C.

Treatment	Storage (Day) 1	7	14	21
Control	5.3	16.0	210.0*	650.0†
Citric acid	4.2	12.0	110.0*	400.0†
Citric acid/200 ppm $Na_2S_2O_5$	3.9	4.0	110.0*	312.0*
Citric acid/350 ppm $Na_2S_2O_5$	1.4	4.8	40.0	64.0
Citric acid/500 ppm $Na_2S_2O_5$	0.7	8.0	14.1	54.0
Citric acid/650 ppm $Na_2S_2O_5$	0.3	2.0	13.0	38.0
Citric acid/800 ppm $Na_2S_2O_5$	0.4	0.8	2.9	42.0
K-sorbate	3.1	3.9	82.0	380.0*
K-sorbate/200 ppm $Na_2S_2O_5$	1.6	3.0	68.0	135.0*
K-sorbate/350 ppm $Na_2S_2O_5$	0.7	1.7	45.0	90.0
K-sorbate/500 ppm $Na_2S_2O_5$	0.2	1.8	12.5	64.0
K-sorbate/650 ppm $Na_2S_2O_5$	0.5	3.0	7.1	81.0
K-sorbate/800 ppm $Na_2S_2O_5$	0.3	1.7	5.2	44.0
Control	5.4	29.0	160.0*	800.0†
Citric acid/K-sorbate	1.9	16.0	7.0	300.0*
Citric acid/K-sorbate/200 ppm $Na_2S_2O_5$	1.1	13.0	43.0	130.0*
Citric acid/K-sorbate/300 ppm $Na_2S_2O_5$	0.7	0.9	12.0	81.0
Citric acid/K-sorbate/400 ppm $Na_2S_2O_5$	0.7	13.0	34.0	62.0
Citric acid/K-sorbate/500 ppm $Na_2S_2O_5$	0.5	5.8	20.0	38.0
Tater white	0.7	5.6	13.0	52.0
STA white	0.3	3.6	4.3	5.3

*Discolored. †Spoiled (slimy, badly discolored, off odor).

antiseptic action of sulfur dioxide towards microorganisms, particularly yeasts, varied with pH, temperature, initial counts, stage of growth, and other factors. With the more acidic solutions, a lower concentration of sulfur concentration was required to inhibit microbial growth (35). As seen from the results (Table 10), with 500 ppm or more $Na_2S_2O_5$ in a citric acid solution, a much lower initial count was obtained. The preservative value of metabisulfite was a temporary effect. It lost its value upon oxidation to sulfate, on volatilization, or combination with other chemical constituents (15). In this experiment, increasing microbial counts were shown during storage time, indicating a decrease in effectiveness.

Potassium sorbate, in contrast to metabisulfite, is more potent against yeasts and molds, but less active against bacteria. The optimal effectiveness of pH can be up to 6.5 with higher activity at lower pH. Reviewing the results obtained, the bonus effect from metabisulfite on inhibition of microorganisms was obvious regardless of the pH values of the cabbage slaw. It is partially because their effect increased about 10-fold when their concentrations raised by a factor of 10 (9).

When citric acid, potassium sorbate, and $Na_2S_2O_5$ were present in the dip solutions, their effectiveness in reducing initial counts was accentuated (Table 10). Three respective initial counts for citric acid treated, potassium sorbate treated, citric acid and potassium sorbate treated packaged cabbage slaw were 4.2×10^5/g, 3.1×10^5/g and 1.9×10^5/g respectively. For citric acid/200 ppm $Na_2S_2O_5$, potassium sorbate/200 ppm $Na_2S_2O_5$ and citric acid/potassium sorbate/200 ppm $Na_2S_2O_5$, the counts were 3.9×10^5/g, 1.6×10^5/g and 1.1×10^5/g respectively.

Tater white, a commercial whitener, not only reduced the initial counts (Table 10) in packaged cabbage slaw but also preserved appearance. The effect of acid, salt, ascorbate, and metabisulfite appeared to be synergistic. Differences in counts from this treatment persisted throughout the entire storage period.

Changes in Oxygen Uptake by Treated Cabbage Slaw During Storage

The determination of residual oxygen in the headspace of the container was originally intended to study the significance of oxygen on the extent of discoloration. Results (Table 11) showed a marked oxygen reduction in the containers of control, citric acid, and citric acid/200 ppm $Na_2S_2O_5$ treated samples. Packaged cabbage slaw is living tissues and should continue to respire in storage under the conditions used in this experiment. Therefore, the suppression of oxygen uptake by chemical treatment might involve changes in the respiratory mechanism resulting from such chemicals.

Citric acid was found to be a respiratory stimulant in castor beans

(36), whereas metabisulfite is known as a reducing agent and has the ability to reduce oxygen tension in plant tissues, to inhibit the enzyme in the TCA cycle (3), and the enzymes in photosynthetic process (24). Nevertheless, the information from this experiment did not involve the accumulation of carbon dioxide along with oxygen depletion which are two parameters in determining the respiration rate of plant tissues. The complexity of the respiratory mechanism alteration by the treatment is beyond this study.

For oxidative browning to occur, molecular oxygen is necessary in addition to PPO and phenols. The higher oxygen uptake by the cabbage slaw might be an indication of the higher oxidation rate occurring in this sample. This assumption was evidenced in the control, citric acid, and citric acid/200 ppm $Na_2S_2O_5$ treated samples with high oxygen absorption rates and intensive discoloration.

The effect of potassium sorbate on the reduction of oxygen depletion was not understandable. It could be due to its antimicrobial func-

TABLE 11.—Change of Oxygen (%) in the Container When Cabbage Slaw Was Stored at 4° C for 21 Days.*

Treatment	Storage (Day)		
	7	14	21
Control	12.06	7.38	2.47
Citric acid	16.27	9.03	2.55
Citric acid/200 ppm $Na_2S_2O_5$	15.57	1.13	1.04
Citric acid/350 ppm $Na_2S_2O_5$	16.30	12.60	9.30
Citric acid/500 ppm $Na_2S_2O_5$	17.67	13.40	12.92
Citric acid/650 ppm $Na_2S_2O_5$	16.37	16.19	15.80
Citric acid/800 ppm $Na_2S_2O_5$	21.96	17.29	17.42
K-sorbate	18.29	14.04	11.59
K-sorbate/200 ppm $Na_2S_2O_5$	19.26	13.59	11.73
K-sorbate/350 ppm $Na_2S_2O_5$	20.85	14.60	12.95
K-sorbate/500 ppm $Na_2S_2O_5$	16.45	12.76	11.96
K-sorbate/650 ppm $Na_2S_2O_5$	16.98	12.76	12.20
K-sorbate/800 ppm $Na_2S_2O_5$	18.67	15.49	13.08
Control	12.06	7.38	2.47
Citric acid/K-sorbate	16.13	11.50	8.77
Citric acid/K-sorbate/200 ppm $Na_2S_2O_5$	15.40	13.29	10.09
Citric acid/K-sorbate/300 ppm $Na_2S_2O_5$	16.63	16.20	15.14
Citric acid/K-sorbate/400 ppm $Na_2S_2O_5$	16.66	14.39	12.52
Citric acid/K-sorbate/500 ppm $Na_2S_2O_5$	16.17	15.52	9.37
Tater white	13.96	13.37	12.88
STA white	14.75	13.80	10.05

*Initial headspace was air (20.2 % O_2).

tion if part of the oxygen depletion observed was the result of microbial respiration (25). However, oxygen depletion in dates accounted for enzymatic browning, seed respiration, and tissue respiration but not mi-

TABLE 12.—Color Scores of Cabbage Slaw by Various Chemical Treatments.*

Treatment	Storage (Day) 1	7	14
		Mean Score	
Control	6.5**	5.8**	
Citric acid	7.7	7.0	
Citric acid/500 ppm $Na_2S_2O_5$	7.9	7.3	
K-sorbate	7.8	7.5	
K-sorbate/500 ppm $Na_2S_2O_5$	8.4	8.0	
Grand mean	7.7	7.1	
Control	7.7	4.3**	discolored
Citric acid/K-sorbate	8.2	6.0	discolored
Citric acid/K-sorbate/200 ppm $Na_2S_2O_5$		7.1	7.6
Citric acid/K-sorbate/300 ppm $Na_2S_2O_5$	8.2	7.1	7.6
Citric acid/K-sorbate/400 ppm $Na_2S_2O_5$		6.6	8.1
Tater white	7.2	8.1	8.1
STA white	8.5	7.6	6.2**
Control (freshly prepared)		8.1	8.1
Grand mean	8.07	7.2	7.7

*A 10-point hedonic scale was used with 10 as perfect and 1 as off.
**Significant at 1%.

TABLE 13.—Total Acceptability Scores of Cabbage Slaw by Various Chemical Treatments.*

Treatment	Storage (Day) 1	7	14	21
		Mean Score		
Control	7.5	5.3**		
Citric acid/K-sorbate	8.1	6.0		
Citric acid/K-sorbate/200 ppm $Na_2S_2O_5$		6.2	6.3	5.0
Citric acid/K-sorbate/300 ppm $Na_2S_2O_5$	6.6	8.1	5.8	5.2
Citric acid/K-sorbate/400 ppm $Na_2S_2O_5$		7.6	5.8	5.1
Tater white	7.1	8.1	5.8	5.8
STA white	4.6**	8.0	7.0	7.1
Control (freshly prepared)		8.0	8.0	7.8
Grand mean	6.7	7.7	6.5	6.0

*A 10-point hedonic scale was used with 10 as perfect and 1 as off.
**Significant at 1%.

crobial respiration (25), because the preservative (ethylene oxide) did not reduce the rate of oxygen depletion.

Sensory Evaluation

Color, flavor, texture, and total acceptability were evaluated by panelists. Results showed a color difference between the control and other treated samples (Table 12). Treatments were effective in maintaining lighter color during storage, with the evidence in accordance with the results obtained from objective measurements (Hunter L).

Judges indicated a flavor inferiority in the sample treated with 500 ppm $Na_2S_2O_5$ in citric acid solution and the sample treated with STA white solution (Table 9). They described it as tart and sour in the former and bitter with undesirable flavor in the latter. This is the drawback of using high concentrations of $Na_2S_2O_5$, even though the color in these samples was very desirable. Neither the treatment nor the storage could change the texture of the product when judged by the panels.

Determination of total acceptability was based on the color, flavor, and texture of the product. Results (Table 13) revealed a decrease in acceptability in all the samples. However, the treated samples were still acceptable after 3 weeks of refrigerated storage. A deviation of the evaluation was observed in STA white treated samples at the first test and the second test. This could be attributed to the fact that the former was based on color and flavor, while the latter was based on color and odor.

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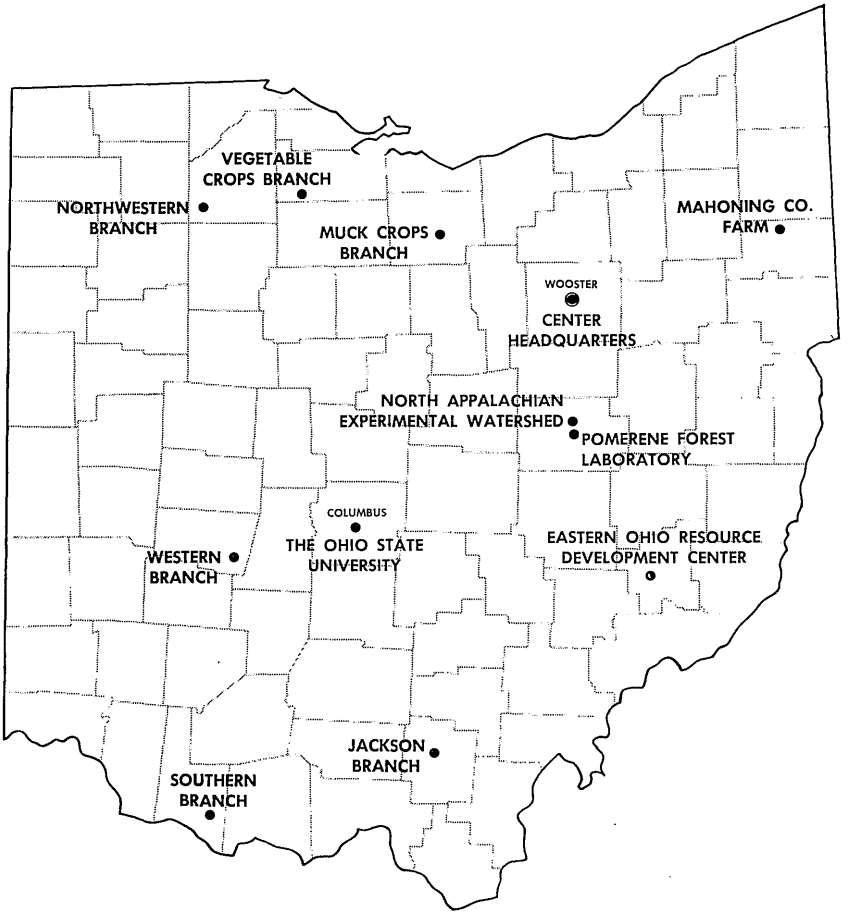

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